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Cytokine Response to Subclinical Cytomegalovirus Reactivation as a Cause of Severe Fatigue in women Undergoing Chemotherapy for Breast Cancer

PRINCIPAL INVESTIGATOR:
Ann Hill

CONTRACTING ORGANIZATION:
Oregon Health and Science University
Portland, OR 97239-3098

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Ann Hill

E-Mail: hillan@ohsu.edu

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Portland, OR 97239-3098

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14. ABSTRACT
We hypothesized that reactivation of the common herpesvirus cytomegalovirus (CMV) might contribute to severe cancer treatment related fatigue (CTRF) in women undergoing chemotherapy for breast cancer. To test this, we carried out a prospective clinical study. 24 women were enrolled and studied before, during and after chemotherapy. Self-reported fatigue levels were recorded, and serum, PBMC and urine samples were cryopreserved for each time point. Cytokine analysis revealed that serum IL-6 levels rose during the course of chemotherapy and that they correlated significantly with fatigue levels. 14 subjects were CMV seropositive, and 10 seronegative. CMV seropositives and seronegatives were not different in age, race, or socioeconomic status. Initial analyses reveal no difference in fatigue or cytokine levels based on CMV serostatus. Thus, preliminary analysis does not confirm our hypothesis. However, some final CMV-related studies of T cell responses remain to be completed, and these may reveal a relationship between CMV reactivation and fatigue in a subset of women. Our study thus supports the theory that chemotherapy induced fatigue is due to inflammation, but does not support a relationship with CMV reactivation.

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Introduction

Cancer treatment related fatigue (CTRF) has a major impact on quality of life both during and after treatment, and the causes are not completely understood. The major aim of this study is to determine whether the activation of cytomegalovirus (CMV) by chemotherapy contributes to the severity of CTRF for women going through chemotherapy treatment for stage I-III breast cancer. The long-term goals of this work are to determine whether CMV reactivation can cause CTRF, to understand the mechanism, to identify patients at risk for CMV-induced CTRF prior to chemotherapy, in order to conduct a clinical trial of anti-CMV drug treatment to prevent CTRF in susceptible individuals. Given the limited scope of this mechanism, the minimal specific goal of this proposal is to determine whether there is sufficient evidence for a role for CMV in CTRF to justify a larger study, and to calculate the size of the study that would be needed to confirm the result. Secondary goals are to determine the associations between CTRF and inflammatory cytokine levels, CMV reactivation, CMV antibody levels, and CMV-specific T cell responses. This study will evaluate fatigue and immune parameters (cytokines and T cells) in equal numbers of CMV+ and CMV- women, 26 in all, undergoing cytotoxic chemotherapy for stage I-III breast cancer. We will study women prior to the start of chemotherapy and at home visits in the weeks between treatments, because this is when fatigue is greatest.

Body

During the 2 year study period, the clinical study was performed as planned, and most of the analyses have been completed, as described below according to the SOW.

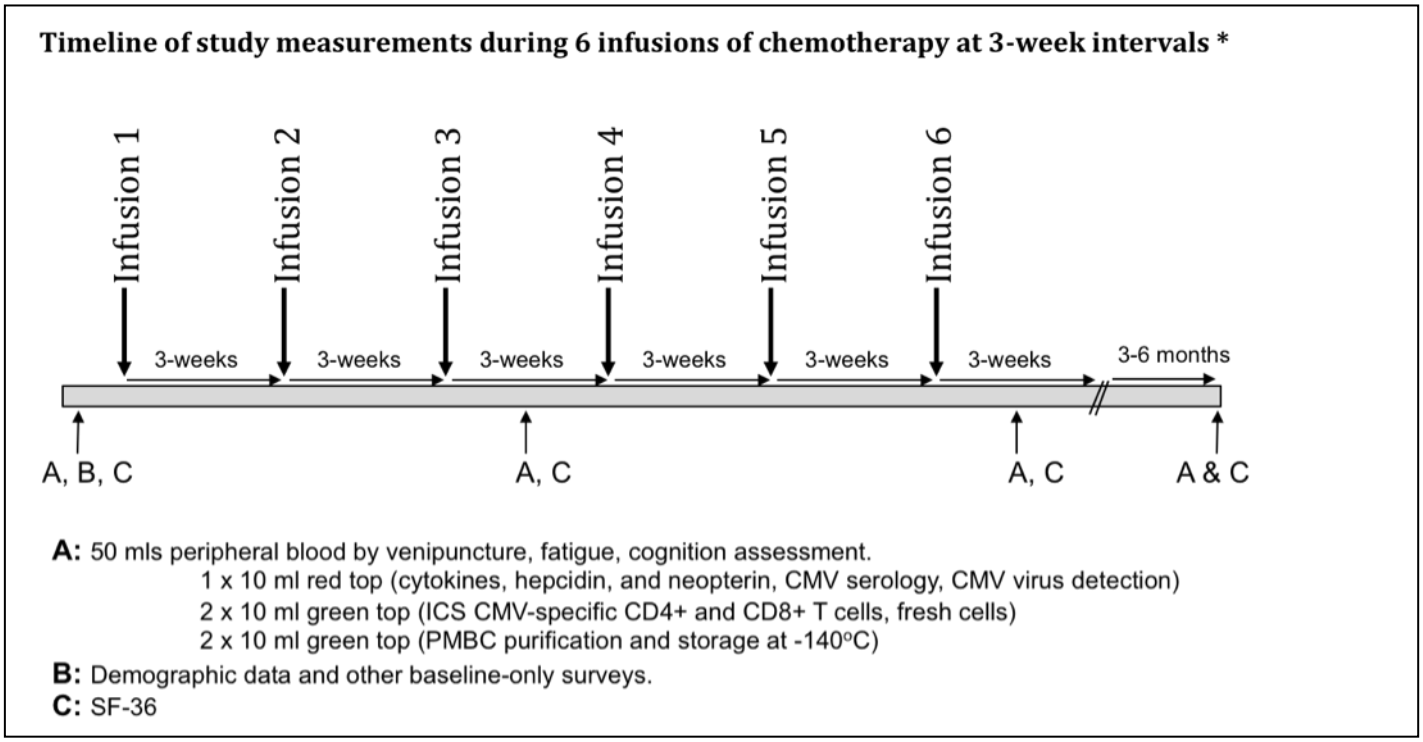
1. Obtained IRB approval for the study including design of study flyer. During the pre award time and first few months of the funding period, the study preparation phase of the project successfully took place and consisted of the following: revisions and approval of the IRB protocol and consent forms; development of standard operating procedures for all protocols; development of recruitment plan for oncologist referrals; development of study materials; and training of Wood study staff.

2. Recruitment. Participant recruitment was delayed for the initial 3 months as the study obtained IRB approval for modifications which included approval of study staff and as we developed processes for referral specific to each of our participating oncologists. After consultation with several referring oncologists, we decided to bring a registered nurse (RN) on to the study team to be able to collect blood samples from participants who had Port-a-catheters (Ports) as a way to minimize any pain and discomfort for participating in this research. It is common practice for breast cancer patients to have a port placed prior to their first chemotherapy infusion so they do not have to have repetitive peripheral blood draws during their course of treatment, and we wanted to be able to provide participants in this study the choice of location for their blood sample collection. The RN on our study staff enrolled in and completed a required research training to update her scope of practice to include port acccess sample collection in August, 2011. Thus, we began official recruitment in September, 2011 through oncologist referrals at the OHSU Marquam Hill hospital and OHSU community oncology clinics. Participating oncologists were sent weekly email reminders of potential breast cancer patients who may be eligible for the study by our study staff who search hospital medical records for oncologist schedules. In addtion, the project coordinator (Torgrimson) attended a weekly oncology meeting to review upcoming patient lists to identify potential study participants. The participating breast cancer oncologists discussed partipation in the study during patient appointments and referred interested persons to our research staff. Upon receiving the patient referral, our study staff contacted the patient to explain study procedures, check eligibility, answer questions, and schedule study visits. Overall, the study was well received by breast cancer patients and the most common reason for refusal to participate was '*feeling too overwhelmed to participate in research during cancer treatment'*.

In order to maximize the number of enrollees, the eligibility criteria were modified to include breast cancer patients who would be receiving trastuzumab in addition to their cytotoxic chemotherapy for their treatment plan. During the first few months of active recruitment, many of the new breast cancer patients being seen at OHSU were not eligibile for our study because their oncologist recommended trastuzumab as part of

their chemotherapy plan. To our knowledge, there is no body of data to indicate that trastuzumab will directly interfere with the outcomes of this study. In light of the fact that there is a growing use of trastuzumab in breast cancer care, the inclusion of these patients allowed our results to inform a broader group of breast cancer patients who have had chemotherapy. By the end of the study period we successfully recruited and completed all study visits for 24 women.

3. Obtain fatigue data, blood and urine at each clinic visit. Additional fatigue data, and blood and urine will be collected twice per treatment cycle (one week after infusion, and one week later) during home visits. Additional fatigue data, blood and urine will be collected from each study participant at the 3 month clinical follow-up appointment with their medical oncologist. Based on IRB regulations and in consultation with oncologists, the initial sampling time point protocol was modified in order to meet regulatory guideline procedures for study participants with cancer who are providing blood samples. According to the OHSU IRB collection procedures, *the amount of blood drawn from an adult may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week*. In order to meet this regulation, we decreased the number of study visits to a total of four ((1) baseline prior to chemotherapy, (2) a mid-treatment during the week following the mid infusion, (3) a final treatment time point during the week follow the last chemotherapy infusion, and (4) a follow up home visit 3 months after the completion of their chemotherapy). A schematic for the revised study visit schedule is below.



* Same timing will be used for participants receiving 8 cycles of chemotherapy

The decision to reduce the number of study visits was made because each visit required 50mls of blood in order to have adequate serum and blood cells to address the primary and secondary aims of this study. Using this modified protocol, we are still address our primary and secondary study aims and test our hypotheses.

Because of the reduced subject burden related to fewer study visits we increased the number of symptoms examined during treatment to include depression, and cognitive difficulties which are also experienced by cancer patients undergoing cancer treatment and which influence the subjective burden of fatigue. In addition we examined quality of life (SF-36) throughout treatment. Using this modified sampling protocol, we have successfully obtained fatigue, depression, cognitive function data, QOL data, peripheral blood and urine for 24 participants at 4 separate time points during treatment.

4. Process and store blood and urine samples on day of collection. All blood and urine samples were successfully processed by study staff in the Hill laboratory on the day of sample collection. Samples were stored at -80 (seum and urine) or in liquid nitrogen (PBMC) n the Wood and Hill laboratories until final analysis.

5. Monthly meetings of both research teams. Dr. Torgrimson held weekly team meeting to discuss the study progress and work with the team on resolving any issues. Meetings were attended by members of the Wood and Hill study teams. In addition, Dr. Torgrimson sent out email updates to both teams to keep all members informed of research decisions made.

6. Determine CMV seropositivity for each patient following first visit. This analysis was completed by the Hill lab. An HCMV ELISA was optimized for this purpose, and anti-CMV antibody level determined for each participant. Fourteen of participants were CMV positive (58%) while 10 were CMV negative (42%).

8. Laboratory analysis of serum cytokines by multiplex immunoassay on pre-chemotherapy infusion blood draw and all blood draws collected at home visit samples. Due to the reduced number of samples collected from each participant we were able to expand on the number of inflammatory analytes measured at each time point.
Serum levels of GM-CSF, IFN-γ, IL-10, IL-12 (p70), IL-13, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, TNF-α, MCP-1, IP-10, IFN-α, IL-1RA, SAA, hs-CRP, adiponectin, leptin, IGFBP-1 and IGFBP-3 were measured in all serum samples by Wood.

9. Measure T cell subsets and CMV-specific T cell responses in CMV seropositives. This analysis has not yet been completed , in part due to a personnel change in the Hill lab, and in part due to the need to improve the protocol. However, it is currently underway. Pilot testing of T cell responses to HCMV pp56 and IE1 peptide pools revealed response that were lower than expected. Consequently, some time was spent optimizing the assay. Specifically, we have found that using the peptides at a higher concentration than recommended by the supplier, and using a customized panel of costimulatory molecules, approximately doubled the number of CMV-specific T cell responses detected by the assay. We are now beginning to analyze the subject samples and expect the analysis to be completed within the next 3 months.

10. Obtain data from clinical records: CBC, relevant clinical history during study period. Cancer diagnosis, staging, chemotherapy type and dosing, additional medication prescriptions, CBC, height and weight were collected at baseline, mid treatment, at the end of treatment and at the 3-6 month follow up where appropriate.

11. Data collation

Torgrimson worked closely with Dr. Leo to collate and clean all study data. Demographic, clinical data, study survey data, and serum cytokine data have been collated into a single SPSS dataset. We are preparing to start the statistical analyses related to Aim 2 of the study which was to determine whether changes in inflammatory cytokine pathways correlate with fatigue and other treatment related symptoms. Additional analyses related to the other aims will be initiated once the CMV analyses have been completed in the Hill laboratory. See Hill Progress Report.

12: Analysis

We hypothesized that cancer treatment related fatigue (CTFR) might be related to subclinical reactivation of the latent herpesvirus CMV, which would stimulate inflammatory cytokines. We predicted that women harboring latent CMV (CMV positives) would have more severe fatigue than CMV seronegatives. To investigate this, we assessed fatigue and inflammatory biomarkers in 24 women undergoing chemotherapy for breast cancer. 14 of out participants were seropositive for CMV, and 10 were seronegative. 23/24 participants were Caucasian. Baseline characteristics of the CMV+ and CMV- participants revealed no significant difference between groups in age (55.3 ± 3.7 and 52.6 ± 3.0 respectively, p=0.601), race, or socioeconomic status.

Women were studied at four time-points (Figure 2), 1) baseline prior to chemotherapy, (2) a mid-treatment during the week following the mid infusion, (3) a final treatment time point during the week follow the last chemotherapy infusion, and (4) a follow up home visit 3 months after the completion of their chemotherapy). At each study visit, participants completed fatigue assessment (Schwartz Cancer Fatigue Scale (SCFS) and the PROMIS-8a), provided a blood sample (10ml plain tube, 40 ml heparinized). PBMC and serum were cryopreserved in multiple aliquots per study visit. An important feature of our study is that the samples obtained during treatment were obtained at home visits a week after the infusion. Analyses are therefore not confounded by the use of corticosteroids in many women on the day of infusion.

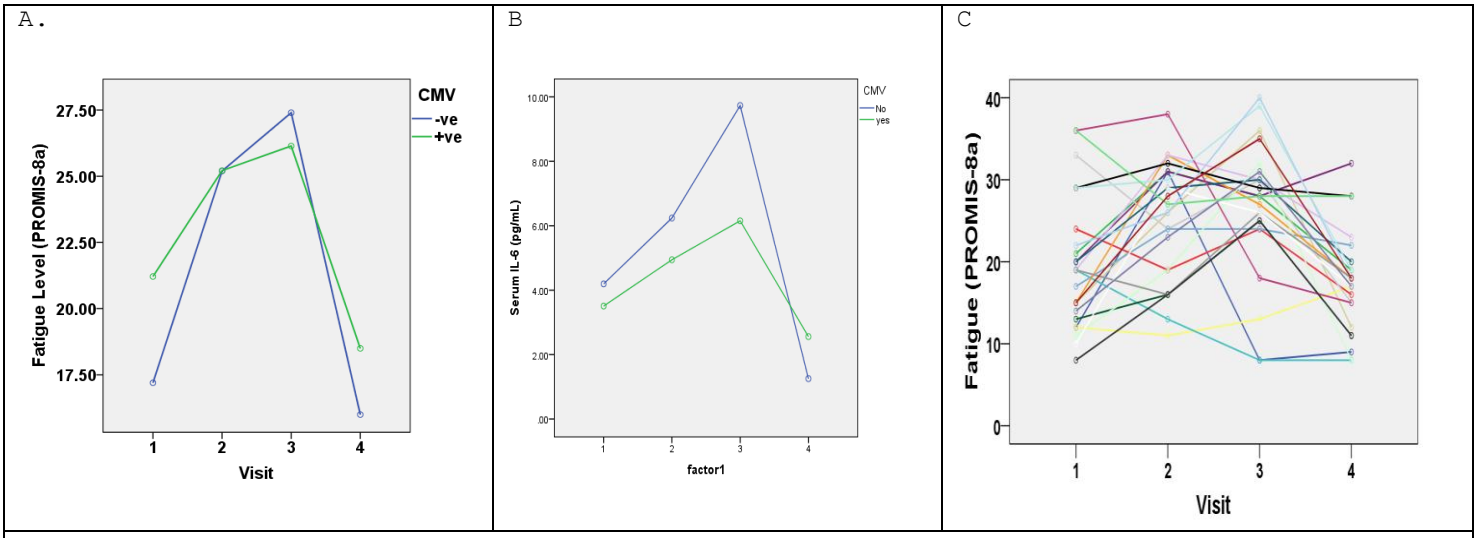


Figure 3: Fatigue and Inflammation during chemotherapy for breast cancer: results of the IDEA award-funded study. A: Self-reported fatigue level at each of the 4 study visits. The mean values for CMV seropositive participants (n=14) and CMV seronegative participants (n=10) are shown. Fatigue increased sequentially during chemotherapy, as expected, but did was not affected by CMV status. B: Mean serum IL-6 levels for CMV+ and CMV- participants over the course of the study. IL-6 levels increased over the course of the study and correlated with fatigue, but were not significantly affected by CMV. C: The fatigue levels are graphed separately for each individual, revealing different patterns in fatigue trajectory among the study participants.

We observed a significant change in fatigue level over the course of the study $F=13.138$, $p<0.001$) Specifically, fatigue levels increased during treatment but declined to baseline levels thereafter. There was no difference in fatigue trajectory between CMV seropositive and seronegative participants ($F=.881$, $p=.456$) (Fig.3A).

We also observed a significant trajectory for serum IL-6 over the course of treatment ($F=3.195$, $p=.029$), also returning to baseline levels. Similarly, there was no observed effect of CMV status on IL-6 levels over time ($F=.490$, $p=.690$).

There was a significant correlation between IL-6 and fatigue during chemotherapy. Our data thus support the inflammatory basis for CTRF.

Although the mean fatigue levels returned to baseline, Fig 3C shows that there were several different patterns of trajectory. Some participants started with very high fatigue scores, likely due to anxiety, and these tended to decrease over the course of the study. Fatigue levels had not returned to baseline levels by the end of the study in a number of participants.

Key Research Accomplishments

- 24 breast cancer patients were enrolled in the study.
- 24 participants successfully completed all study visits.

- Fatigue, depression, cognitive function, and quality of life data were collected 4 times during treatment: before the start of chemotherapy, after the 1st infusion, after the mid-point chemotherapy infusion, after the last infusion and then at a follow up visit 3-6 months after the final infusion.
- Peripheral blood for serum and PBMC preparation, and urine were collected from all study participants at each time point.
- Clinical data including medications, height and weight, CBC etc. were collected from the clinical record.

- Serum levels of GM-CSF, IFN-γ, IL-10, IL-12 (p70), IL-13, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, TNF-α, MCP-1, IP-10, IFN-α, IL-1RA, SAA, hs-CRP, adiponectin, leptin, IGFBP-1 and IGFBP-3 have been measured in all serum samples.
- Demonstration that fatigue correlated with serum IL-6.
- Preservation of samples in a biorepository and obtaining consent from subjects for use in future studies

Reportable Outcomes

This study has not yet generated publications. However, based on our preliminary analysis above, we expect to prepare two publications: one describing the inflammatory measures and their relationship to fatigue, and another describing the impact of chemotherapy on CMV T cells and activity**.**

Conclusions

This study did not support our original hypothesis regarding CMV reactivation. However, participants have given consent for their samples to be stored in a repository and used in future studies, creating a valuable resource which we now intend to exploit in further studies. It is also possible that the CMV T cell studies will provide evidence of CMV reactivation in a small subset of women. If so, we might find that that correlates with fatigue and/or inflammatory outcomes: such a result would warrant futher exploration.

References

N/A

Appendices

There are no supplementary appendices to include at this time, but any study document will be provided as requested.

Supporting Data

None at this time.